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Serum and tissue concentrations of doxorubicin after IV administration of doxorubicin or doxorubicin-DNA complex to patients with gastrointestinal cancer*

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Summary. Blood and tissue concentrations of doxorubicin (DOX) were assayed after an intraoperative IV test dose of either free DOX 10 mg or its DNA complex 10 mg to patients with gastrointestinal cancer. After administration of the free drug, blood DOX levels decreased in an at least biphasic way, while DOX–DNA gave higher blood concentrations, which decreased slowly with no clear inflexion point on the concentration-time curve within the first hour. Tissue concentrations of DOX did not differ significantly after the two forms of the drug, liver being the tissue with the highest levels, followed by lymph node metastases, tumor tissue, muscle, and normal intestinal mucosa. If skeletal muscle can be used as a substitute for myocardium, lower cardiotoxicity of DOX–DNA than of DOX is not likely to be due to a difference in tissue uptake and retention between the two forms of DOX.

Introduction

Long-term doxorubicin (DOX) treatment carries the risk of serious cardiotoxicity. To circumvent this limitation, modified treatment intervals, dosages, and/or infusion rates have been suggested.

Attempts have also been made to target anthracyclines by linking them to carriers that could selectively increase the drug uptake in tumor tissue. One carrier studied is dextran [10] and another is DNA [18]. The anthracyclines have a high affinity to DNA, which being a macromolecule enters the cells by endocytosis. It has been suggested that this process is more active in tumor cells than in myocardium [18]. In the cells, lysosomal enzymes are thought to digest the carrier, leaving free drug. Recent results suggest, however, that anthracycline– DNA complexes may dissociate to a great extent while still in the blood, thereby acting as slow-release preparations [8, 14].

Anthracycline – DNA complexes have been used to treat a variety of neoplasms (review: [17]). In general, antitumor activity was retained, while cardiotoxicity seemed reduced [6, 12]. However, endomyocardial biopsies from DOX – DNA-treated patients had morphological changes similar to those seen after treatment with DOX in the free form [4].

The present study concerns serum and tissue concentrations of DOX after intraoperative systemic administration of free or DNA-bound drug to patients with gastrointestinal cancers. Blood, tumor, skeletal muscle (as a substitute for myocardium [11]) and, when possible, other appropriate tissues were sampled.

Materials and methods

Patients and sampling. Patients with gastrointestinal cancer received an intraoperative IV 10-mg-test dose of DOX in the free form (10 cases) or bound to DNA (9 cases). DOX (Adriamycin, Farmitalia Carlo Erba) was dissolved in 5 ml physiological saline and infused over 2–5 min or mixed with 50 ml freshly autoclaved herring sperm DNA (type VII, Sigma Chemicals, 2.34 mg/ml). The mixture was incubated at room temperature for at least 20 min prior to rapid (5–10 min) infusion. The end of the infusion was used as a reference time point (0 min).

Usually, serial samples of serum and abdominal muscle were obtained. A non-necrotic piece was taken from the resected tumor specimen and the time when it was completely devascularized was considered as the sampling time. As a rule, normal adjacent mucosa was also sampled. Normal liver and metastases in different locations were occasionally biopsied. The samples were stored at −20 °C.

Drug assay. Serum concentrations of DOX and its reduced metabolite doxorubicinol were determined by HPLC [14]. Once the tissue specimens had been thawed and rinsed 2 ml 0.1 *M* phosphate buffer, pH 8.1, and 100 µl 10 µ*M* daunorubicin were added to 0.5–1 g tissue. The tissue samples were homogenized with two 5-s pulses using a Kinematica polytron at setting 5, followed by sonication for 30 s. The drug analysis was then performed as for serum samples.

Results

Sera

The serum concentrations of DOX after its administration as the free drug or the DNA complex are shown in Fig. 1. DOX – DNA gave higher drug levels than the free drug.

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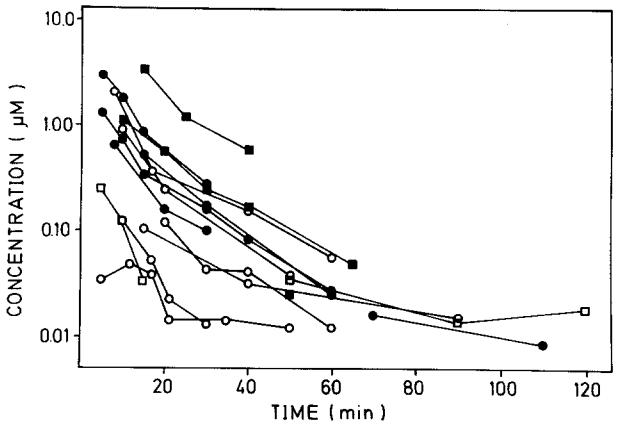

Fig. 1. Time course for the serum concentration of DOX after an IV injection of DOX in the free form *(open symbols)* or complexed to DNA *(filled symbols). Circles* indicate patients with normal liver function tests and *squares*, patients with at least one abnormal liver function test

The initial steep decline in concentration after free DOX seemed to reach an inflexion point at about 20 min after administration, while a second phase was not evident after DOX – DNA. Two patients receiving free DOX, and three given DOX – DNA had gross liver metastases and abnormal liver function test results. As seen in Fig. 1, their drug concentration curves did not differ strikingly from those of the other patients. Doxorubicinol in serum was detected neither after DOX nor after DOX – DNA.

Tumors. Free DOX gave tumor concentrations ranging from 0.13 to 1.5 nmol/g in six patients 60–100 min after drug administration. There was no obvious trend when levels were plotted against time, except that serial biopsies of a carcinoid tumor at 80, 90, and 100 min had DOX levels of 1.5, 1.3, and 0.21 nmol/g, respectively. One patient with liver metastases had a drug concentration in the middle range, 0.30 nmol/g, in the primary tumor at 100 min. The mean tumor drug level after free DOX was 0.60 nmol/g.

DOX – DNA yielded tumor drug concentrations in the range of 0.36–1.2 nmol/g at 10–120 min after injection. This procedure also led to no evident time trend. The mean tumor drug concentration after DOX – DNA was 0.75 nmol/g, not significantly different from that after free DOX ($P > 0.05$).

Metastases. By 15–60 min after the injection of free DOX to one patient, three serial biopsies of omental tumor deposits contained between 0.46 and 0.55 nmol DOX/g, slightly more than the corresponding muscle samples. A liver metastasis in another patient had a drug level of 0.39 nmol/g at 25 min after free DOX. At this time, muscle contained 0.075 nmol DOX/g. In neither case was the primary tumor sampled.

Lymph node metastases happened to be biopsied only in cases receiving DOX – DNA. In one patient, the DOX concentrations in lymph node metastases were 1.85 nmol/g at 15 min and 2.40 nmol/g at 45 min, as against muscle levels of 0.32 and 0.75 nmol/g at the same times. No sample of the primary tumor was available. Another patient had drug concentrations of 0.93 nmol/g at 25 min in the node metastasis, 0.58 nmol/g at the same time in the primary colonic tumors and 0.27 nmol/g 10 min later in striated muscle. A third patient had DOX concentration of 0.62 nmol/g in the node deposits at 30 and 40 min after DOX – DNA, 1.20 nmol/g in the primary cecal tumor at 40 min, and 0.61 nmol/g in skeletal muscle at 30 min.

Normal liver. After free DOX, three patients had liver concentrations of 3.7, 3.0, and 2.6 nmol DOX/g at 30, 80 and 110 min, respectively. After DOX – DNA, one patient had a level of 3.3 nmol/g at 20 and 50 min and another had 3.9 nmol/g at 60 min.

Skeletal muscle and smooth muscle. Abdominal striated muscle contained between 0.043 and 0.41 nmol DOX/g within the first 2 h after administration of the free drug (Fig. 2a). Individually typical levels were often reached within 10–20 min. However, both increasing and decreasing concentrations were found in serial biopsies from different individuals within the same time interval. In one DOX-treated case, striated muscle had a drug level of 0.21 nmol/g at 55 min, while smooth muscle (myometrium) contained 0.07 nmol/g at this time.

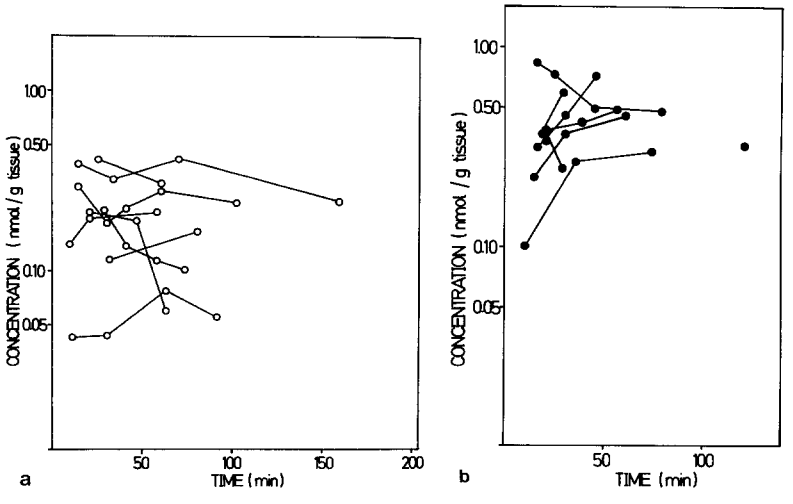

Fig. 2a, b. Time course for the concentration of DOX in skeletal muscle tissue after an IV injection of 10 mg DOX, in the free form **(a)** or complexed to DNA **(b)**

DOX – DNA gave muscle concentrations of DOX between 0.11 and 0.85 nmol/g within the first 2 h (Fig. 2b). In five of seven cases the levels increased during the first 30–60 min, while in one case the DOX concentrations steadily decreased in four samples 15–80 min after the infusion, from 0.85 to 0.49 nmol/g.

Ratios of tumor DOX concentration to normal tissue DOX concentration. After DOX administration the individual ratios of the drug concentration in the tumor to that in adjacent normal mucosa ranged from 0.5 to 2.0, with a mean of 1.1. After DOX – DNA the range was 0.3–2.2, with a mean of 1.3 ($P > 0.05$).

Similar ratios for tumor DOX to muscle DOX concentrations were calculated. Actual or intrapolated values from the muscle concentration curves were used. The ratios did not change with time (correlation coefficient for ratio vs time after both free DOX and DOX – DNA were -0.17, $P > 0.05$). Therefore, group mean ratios were compared with recourse to Student's *t*-test. The mean ratio after free DOX was 3.5 ± 1.8, and that after DOX-DNA was 2.2 ± 0.5 ($P > 0.05$).

Discussion

We compared serum and tissue concentrations of DOX after its IV administration in free form or bound to DNA. Serum DOX concentrations after administration of the free drug seemed to follow known distribution and elimination phases, with a short and a longer $t_{1/2}$ [3, 5, 7]. The low dose of DOX we used may still allow comparisons with other studies, since the initial $t_{1/2}$ for DOX seemed unrelated to the drug dose [15]. Our observation time was too short for the detection of a possible third phase [5, 7].

DOX – DNA gave higher serum drug concentrations than the free drug. This is compatible with the findings of others workers, even though in their studies prolonged infusions of anthracycline – DNA were given [1, 2, 8].

Two patients receiving free DOX and two given DOX – DNA with liver metastases did not differ from the nonmetastatic cases in their serum drug levels after DOX. The relevant literature is sparse and somewhat conflicting. Some correlation was found between the extent of liver involvement and slower plasma decay of DOX in one study [9], while another study was reported to show normal DOX clearance in the presence of a liver tumor [5].

The DOX concentrations differed considerably between various tissues. Liver had high levels after both free and complexed DOX, as previously reported for free drug [5, 9]. Tumors had higher DOX levels than muscle after both free DOX (tumor/muscle ratio 3.5) and DOX – DNA (ratio 2.2). The difference was not statistically significant and seemed mostly related to a difference in muscle drug levels. Tumor tissue had completely overlapping drug concentration ranges after the two forms of the drug, as did normal liver. Metastases in patients receiving DOX – DNA were by chance represented only by lymph node deposits and no such was sampled after free DOX. They often had higher drug levels than the nonlymphatic metastases sampled after free DOX. Normal lymphatic tissue had a higher drug concentration than tumors after free DOX [5, 9], presumably due to its high DNA content [16]. The high DOX levels in lymph node metastases may therefore be caused by persisting lymphatic tissue.

We could not detect doxorubicinol in any sample, even though our assay readily identifies this metabolite. This can be explained by the low test doses used in the present study.

Our study showed wide interindividual variation in the pharmacokinetics of DOX in serum and tissues. This could not be explained by differences in body surface area or kidney or liver function. Therapy with standardized DOX doses therefore seems to lack sophistication, so that further pharmacokinetic analyses correlated to therapeutic and side effects seem to be indicated.

The results do not support the hypothesis that tumor DOX uptake and retention can be selectively enhanced by administering the drug as a DNA complex. As an alternative theory, the prolonged high serum drug concentrations after DOX – DNA may be related to a lower immediate DOX uptake by tissues. This may possibly explain the claimed reduction of DOX cardiotoxicity by its binding to DNA.

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